STANDARD OPERATING PROCEDURE

BLOOD ALCOHOL ANALYSIS (METHOD 2)
(Revised 7/26/02)

Principle:

Headspace chromatography is based on Henry's Law which states, for dilute solution, the solubility of a gas in a liquid expressed as a mole fraction depends upon the pressure of the gas. There is a fixed ratio between the mole fraction of the gas in the air and the mole fraction in the liquid. This ratio remains constant for a given temperature.

Specimens:

Optimum sample volume - 3 ml or greater.
Samples which contain less than 3 ml may be analyzed.

Acceptable specimens are whole blood, urine, or other alcoholic solutions. Other specimens may be analyzed with the approval of the laboratory director.

Blood: The preferred sample is blood that is submitted in the State Toxicologist BAC Kit. These kits have sterile vacutainer tubes containing sodium fluoride and potassium oxalate.

Urine: The preferred sample is urine that is submitted in the State Toxicologist Urine Kit.

Refrigerators may be used for specimen storage. Specimens should be kept at 4°C.

Equipment:

1. Gas Chromatograph

    1) FID detector or equivalent for volatiles
    2) Column temp. = 85°C
    3) Carbopak B column packing or equivalent column packing for volatile system
    4) Stainless steel, nickel, or glass capillary, or equivalent column
    5) Gas flows: N_2 = 50 ml/min
    H_2 = 40 ml/min
    Compressed air = 600 ml/min

6) Injector Temp. = 250°C
7) Detector Temp. = 250°C

2. Headspace autosampler

Platen temperature: 60°
Platen equilibration: 10.00 min
Sample equilibration: 15.00 min
Vial size: 20 ml
Mixer: ON
Mix: 1.00 min
Mix power: 2
Stabilize: 1.00 min
Cap cooldown: NI
Min at: NI
Pressure: 0.50
Pressure Equilibration: 0.25 min
Loop fill: 0.15 min
Loop Equilibration: 0.05 min
Inject: 0.30 min
Cap inject: NI
Min at: NI
Valve: 120°C
Line: 125°C
Capillary Union Heater: NI
Injection per vial: 1
GC cycle time: 5.00 min
Parameter optimization: OFF
NI
Sweep gas: 800 ml/min

3. Integrator, recorder, scientific calculator, and/or gas chromatography data system

Run parameters
Zero - 0
ATT 2^ - 1
CHT SP - 0.5
AR REJ - 10
THRSH - 0
PK WD - 0.07

Timetable events
0.000 INTG # 8
0.000 INTG # 9

0.000 INTG # -9
4.700 STOP

(NOTE: Temperatures, pressures, and other parameters in 1., 2., and 3. are suggested operating conditions and may need to be altered to obtain optimum chromatographic results.)

4. Vials, caps, and septums

5. Other laboratory supplies

    1) pipettes (calibrated) (SMI and/or equivalent)
    2) automatic delivery pipet
    3) weighing bottles and lids
    4) volumetric flasks and stoppers (various sizes)
    5) analytical balance
    6) polyethylene bottles (500 ml)
    7) polyethylene microcentrifuge tubes with caps (500 ul)
    8) 5 ml beakers
    9) pasteur pipets
    10) stock solutions
        1) blood (blank blood treated with NaF to a concentration of 10 mg/ml)
        2) urine
        3) alcohol (either pure alcohol or the 95.6% azeotrope)
        4) n-Propanol

Reagents:

1. n-Propanol, $CH_3CH_2CH_2OH$, analytical grade. **Flammable, may be harmful if swallowed, inhaled, or absorbed through the skin.**

2. Ethanol, CH_3CH_2OH, 200 proof, dehydrated USP grade. **Flammable, may be harmful if swallowed, inhaled, or absorbed through skin.**

3. Sodium fluoride, NaF, analytical grade. **May be fatal if inhaled or swallowed.**

4. Sodium hydrosulfite, $Na_2S_2O_4$, analytical grade. **Flammable. May ignite with moisture and air. Harmful if swallowed. Causes irritation.**

5. Ammonium sulfate, $(NH_4)_2SO_4$, analytical grade.

6. Ethanol azeotrope, 95.629 $\pm$ .028 percent by weight, National Institute of Standards and Technology, United States Department of Commerce. Stored at 4°C. Expiration date determined by manufacturer.

7. Ethanol Calibrators: Made with 200 proof USP grade ethanol and deionized water. Store in the refrigerator at 4°C in 500 ml plastic bottles. Expiration date is 2 months from date of initial use.

8. Diluent: Made with analytical grade ammonium sulfate, analytical grade sodium hydrosulfite and deionized water. Store at room temperature in one liter glass volumetric flask. Expiration date is 2 months.

9. Internal Standard: Made with analytical grade n-propanol and diluent. Store at room temperature in a one liter glass volumetric flask. Expiration date is 2 months.

10. Blood Bank: **Use Universal Precautions when handling biohazardous material.** Prepared by purchasing whole blood from United Blood Services of Bismarck and adding analytical grade sodium fluoride. Expiration date is 4 months. Store in refrigerator at 4°C.

11. Urine Blank: **Use Universal Precautions when handling biohazardous material.** Prepared by mixing sodium fluoride with urine obtained in-house. Expiration date is 4 months. Store in refrigerator at 4°C.

12. Aqueous Commercial Controls: concentration range 0.10 g% and 0.30 g% ethanol by weight. Expiration date determined by manufacturer. Store at room temperature.

<u>Preparing Standard Ethyl Alcohol Solutions</u> : (See Table I)

1. Use calibrated SMI pipets (or equivalent).

2. Use pure anhydrous ethyl alcohol or ethanol azeotrope, 95.629 ± .028 percent by weight.

3. Fill the appropriate size volumetric flask to about 4/5 full with distilled water.

4. Place approximately 10-15 ml of distilled water into weighing bottle, fit with lid, place on analytical balance and tare.

5. Deliver the required volume of pure anhydrous alcohol to the weighing bottle and note the weight in grams to 3 significant figures.

6. Quantitatively transfer the contents of the weighing bottle into the appropriate size volumetric flask, rinsing the weighing bottle several times with distilled water.

7. Fill the volumetric flask to the mark with distilled water and mix contents thoroughly

8. Transfer into 500 ml polyethylene bottles and store in refrigerator at 4°C.

9. Check new standards against the previous standards for accuracy. Run in triplicate. Standards <0.10 g% must be within ± .005% of weighed value, while standards > 0.10 g% must be within 5% of weighed value.

TABLE I

Ml of Pure Alcohol	Volumetric Flask* Size	Standard Solution Concentration
0.324	1 L	0.025 g% ± .002 g%
0.324	0.5 L	0.050 g% ± .004 g%
0.960	0.5 L	0.150 g% ± .005 g%
2.220	0.5 L	0.350 g% ± .005 g%
3.500	0.5 L	0.550 g% ± .005 g%

These solutions will be used in preparing the calibration curve.

Preparation of Diluent and Internal Standard Solutions:

1. The diluent solution is prepared by dissolving 132 grams of ammonium sulfate and 17.4 grams of sodium hydrosulfite per liter of distilled water.

2. The internal standard solution is prepared by diluting a weighed volume (250 ul) of n-propyl alcohol per liter of diluent solution to obtain a concentration within the range of 0.018 g% to 0.022 g%.

Preparation of Volatiles Solution

1. The "volatiles" solution is a dilution of 25 ul each of methanol, acetone, ethanol, isopropanol and n-Propanol into a 100 ml volumetric flask.

2. The flask is approximately half filled with distilled water before the addition of the various volatiles and then filled to the mark with distilled water.

3. Invert several times to mix contents.

4. This solution is for qualitative use only.

Preparation of Standards, Controls, Case Samples, Blank, and Zero for Analysis

1. Table II summarizes the preparation of each required item for analysis. Table III shows the procedure for preparing the calibrators.

TABLE II

	Volume Used	Amt of Blood Added	Amt of Dist Water Added	Amt of Diluent Solution	Amt of IS Solution
Standards	100 ul	100 ul	---	---	2 ml
Commercial Control(s)	100 ul	100 ul	---	---	2 m.
Blank	---	100 ul	100 ul	2 ml	---
Zero	---	100 ul	100 ul	---	2 ml
Volatiles	100 ul	100 ul	---	---	2 ml
Sample	--	100 ul	100 ul	--	2 ml

2. Each standard ethyl alcohol solution is prepared in singlet. Blank, zero, and volatiles are prepared in singlet.

3. In-house and commercial controls are prepared either in singlet, duplicate, or triplicate depending on the number of case samples to be run. Case samples are prepared in duplicate.

4. Once all components are placed in the labeled vial, it is capped and crimped.

TABLE III - Standard Curve Preparation

Standard Concentration	Amt.Etoh Added	Water	Blank Blood	IS Soln
0.025 g%	100 ul	0	100 ul	2 ml
0.050 g%	100 ul	0	100 ul	2 ml
0.150 g%	100 ul	0	100 ul	2 ml
0.350 g%	100 ul	0	100 ul	2 ml
0.550 g%	100 ul	0	100 ul	2 ml

Sample Loading Procedure

1. A worksheet is then prepared indicating the position of each vial in the carousel of the autosampler. The worksheet should be rechecked before starting the analysis.

2. Upon completion of the analysis, the position and identity of the vials should be compared to the chromatogram to verify the injection sequence prior to removal of the vials from the carousel.

3. The proper sequence for beginning an alcohol analysis would be to run the 5 ethyl alcohol standards, then the blank, zero, and volatiles solutions, followed thereafter by a constant pattern of a control, case sample (in duplicate), and a control.

4. When running the standard curve, allow the instrument to run the first standard and then stop. This will allow the analyst sufficient time to input all necessary information into the integrator. Then restart the instrument and allow the remaining four standards to be analyzed. Input the appropriate information after each standard has been analyzed and before the next is injected.

5. The number of controls analyzed should not be less than 25% of the case samples being tested. If more than 14 case samples are to be run, vials that have already been analyzed and checked against the chromatograms from the integrator may be removed and a continuing pattern of case sample (in duplicate) and a control may be placed onto the autosampler carousel.

6. As long as the controls continue to be acceptable, there is no need to rerun the standard curve. However, if any control falls out of acceptable range then the case sample prior to and immediately following it must be reanalyzed.

7. Once the standard curve is completed and while the blank, zero, and volatiles are being analyzed, the correlation coefficient of the curve should be calculated. If it is not greater than or equal to 0.999, then the standard curve should be rerun.

8. If a case sample falls outside the range of the standard curve or it is initially deficient in quantity, it may be diluted and then analyzed. The tested value will then be adjusted according to the amount of the dilution.

Manufacturers and vendors for chemicals, reagents, and supplies include, but are not limited to:

Quantum Chemical Corporation
Criterion Sciences
Fisher Scientific

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26 July 2002